Investigation of the Protease Domain of the Lacticin 481 Transporter, LctT

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Lacticin 481 is a lanthionine-containing antibiotic (lantibiotic) produced by the Gram-positive bacteria Lactococcus lactis subsp. lactis. Lantibiotics are cyclic peptide antibiotics that are ribosomally-synthesized as precursor peptides and post-translationally modified to give rise to the mature species. The final steps of lantibiotic biosynthesis include export and cleavage of an unmodified N-terminal leader sequence to reveal the biologically active peptide. LctT is the transporter that both cleaves the leader sequence from the lacticin 481 propeptide LctA and exports the mature lantibiotic. LctT belongs to the recently described family of AMS (ABC transporter maturation and secretion) proteins whose prepeptide substrates share a conserved double-glycine type cleavage site. While the *in vitro* activity of non-lantibiotic bacteriocin proteases LagD and ComA have previously been described, the activity of a lantibiotic protease has not yet been characterized. Herein, we report the purification and *in vitro* activity of the protease domain of LctT. The G(-2) A(-1) cleavage site and several other conserved amino acid residues were targeted for site-directed mutagenesis to generate LctA mutants that probe the substrate specificity of LctT. While the conserved leader sequence residues do not seem to play a role for the LctA modification enzyme LctM, they are important for the LctT-catalyzed cleavage reaction. Finally, the protease domain of LctT was shown to be a cysteine protease through site-directed mutagenesis of Cys 12 and His 90.